

Micropropagation of *Saintpaulia* at Singapore Botanic Gardens

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Abstract

Experimental propagation of *Saintpaulia* by means of leaf and petiole culture was conducted at the Singapore Botanic Gardens. All in, 17 varieties were used. Leaf culture of 15 varieties and petiole culture of 3 were successful. Shoots were found to initiate readily in a large variety of media but satisfactory rooting occurred only in media with a low level of growth regulators such as IAA. After the plantlets were transferred into soil, it was found necessary to keep them in an enclosed environment for about two months in order to ensure a high survival rate.

Introduction

Saintpaulia is commonly propagated vegetatively from leaf cuttings by amateurs and commercial growers. Although new plants can be grown from leaf cuttings within 6–8 weeks, the number of plants per crop that can be obtained by this method is small. This method is therefore not satisfactory for commercial mass production.

Tissue culture of *Saintpaulia* has been successfully attempted in western countries (Kukulozanka and Suczynaka 1972, Start & Cumming 1976, Bilkey et. al. 1978). Their reports gave fairly detailed accounts of the initiation of shoots and roots in culture. However, very little information is available on the technique of transplanting plantlets from flasks into the open.

The Singapore Botanic Gardens started experiments on tissue culture of saintpaulias about 4 years ago. So far 15 varieties have been successfully propagated by this technique and seven of them are now being mass-propagated at the laboratory. Our experiments and technique are described in this report.

Micropropagation of *Saintpaulia*

(a) Method

Fully expanded young leaves with a petiole 3–4 cm long were used. The leaves were washed in soap water, then soaked in detergent for 15 minutes to remove dirt, thereafter sterilized by soaking in 10% clorox solution for 10–20 minutes, and finally rinsed several times with sterilized distilled water.

After disinfection, the leaves and petioles were cut apart and then each sliced into 3 portions. The leaves were sliced such that each segment carried a section of the midrib.

Murashige and Skoog's formula (1962) was used with the addition of the following growth regulators:

- (1) 2.5 mg/l kinetin, 5 mg/l IAA (AS)
- (2) 0.3 mg/l NAA, 0.1 mg/l kinetin, 40 mg/l adenine sulphate (ASP)
- (3) 0.1 mg/l NAA, 0.01 mg/l BA (AF)
- (4) 0.2 mg/l BA, 0.1 mg/l zeatin (WSK6)
- (5) 2 mg/l BA, 1 mg/l zeatin (WSK3)
- (6) 2 mg/l IAA, 2 mg/l kinetin (AB)

The explants were cultured on 20–30 ml of the medium in conical flasks or on 10–20 ml of medium in test tubes. Each leaf segment was placed upright in the culture with one quarter of its length embedded in the agar. The cultures were exposed to Gro-lux lighting for 10 hours a day and maintained at an environmental temperature of 20–25°C. Two tubes, each of 30 watts were positioned a foot above the bench.

(b) *Results of leaf culture*

A total of 15 different varieties of *Saintpaulia* had successfully proliferated through leaf culture (Table 1). After about 3 weeks, tissues of leaf explants began to enlarge and thicken. Shoots first appeared at the base of the midribs, touching the medium and rapidly spreading to the entire adaxial area of the blade. Although all explants were placed upright in the medium, only a few shoots formed on the abaxial surface of the lamina and these were mainly located around the midrib. After 1–2 months the whole flask was crowded with shoots, initiated from the leaf explants and the culture was then ready for transfer.

(c) *Results of petiole culture*

In petiole culture, swelling of the petiole was often observed around the circumference. In most cases, the central portion at the cross-sections showed no growth and gradually browned off. After 1–2 weeks, callus growth began. Most of the petiole cultures formed callus alone or with roots. About 20% of the cultures formed shoots (Table 1). Plantlet regeneration became noticeable after 6 weeks.

The cultures of petioles were much less successful than those of leaves. So far only 3 varieties differentiated plantlets from cross-sections of excised petioles in vitro.

Shoot multiplication and root formation

After about 2 months, the shoots had to be transferred (Plate 1). Shoots taller than 1 cm were separated with forceps and placed on a rooting medium which consisted of Murashige and Skoog's medium plus 0.1 mg/l IAA. Those shorter than 1 cm were subcultured for multiplication. The medium for multiplication was also Murashige and Skoog's but with 0.5 mg/l kinetin and IAA.

On the multiplication medium, these plants developed into a compact spherical mass of multiple shoots in 6 weeks. Where the shoots had not been subcultured after 3 months, some became dominant, suppressing the growth of the others.

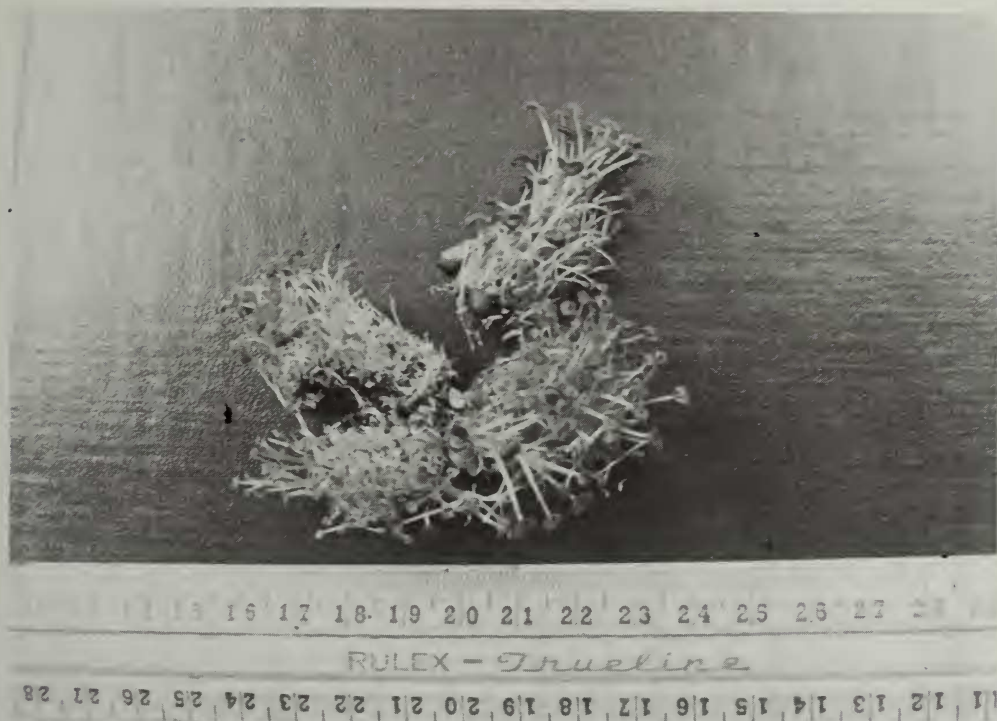


Plate 1. Leaf-cultured *Saintpaulia*, P1, with multiple shoots.



Plate 2. Leaf-cultured *Saintpaulia*, P1, ready for transplanting.

Table 1
***Saintpaulia* varieties successfully propagated by tissue culture technique**

Departmental Code	Description	Proliferation media	Explant	Proliferation time	Formation of plantlet
	WHITE VARIETIES				
W1	White (R.H.S. 155D), double petals, light purplish centre; entire or serrated leaves.	AS AB	leaf	2 weeks	+++
W2	White (R.H.S. 155B), double petals, leaves with slightly indented margins.	ASP	leaf petiole	3 weeks 5 weeks	+++ ++
W3	White (R.H.S. 155D), double petals, trailer; entire, pointed leaves.	AS	leaf	6 weeks	+
	PINK VARIETY				
P1	Rhodamine Pink (R.H.S. 62A, H.C.C. 527), double petals; entire or serrated leaves.	AS AB	leaf petiole	4 weeks 5 weeks	+++ ++
	BLUE AND VIOLET VARIETIES				
B/P1	Gertian blue (R.H.S. 96B), double petals; entire, rounded leaves.	AB	leaf	3 weeks	+++
B/P2	Violet-blue (R.H.S. 89A), single petals; entire or serrated leaves.	AC	petiole leaf	6 weeks 6 weeks	++ ++
B/P3	Violet-blue (R.H.S. 89B), single petals; velvety texture; entire leaves.	AB	leaf	5 weeks	++
B/P4	Violet-purple (R.H.S. 77A, H.C.C. 733) double petals; entire leaves.	AB	leaf	4 weeks	+++
B/P5	Violet-purple (R.H.S. 77A, H.C.C. 733), single star-shaped petals; entire or serrated leaves.	AS	leaf	4 weeks	++
	VARIEGATED FOLIAGE				
VF1	Pink (R.H.S. 73B), double petals; Tommie Lou's foliage.	AS	leaf	3 weeks	++
VF2	Roseine Purple (R.H.S. 68C, H.C.C. 629/2), single petals; Tommie Lou's foliage.	ASP	leaf	3 weeks	+

Table 1 (cont.)
Saintpaulia varieties successfully propagated by tissue culture technique

Departmental Code	Description	Proliferation media	Explant	Proliferation time	Formation of plantlet
TC1	TWO-TONE AND MULTICOLOUR Solferino Purple (R.H.S. 65B, H.C.C. 26/3) with Spiraea Red (R.H.S. 63C, H.C.C. 025/2) radiating from the centre and bordering the edge, single fringed petals; big and heavily rippled leaves, almost bordering on fringe.	AF	leaf	5 weeks	+
TC2	Pink (R.H.S. 62D) double petals, with good white edge; entire, pointed leaves.	AC	leaf	2 weeks	+
TC3	Violet-blue (R.H.S. 93B) with narrow white edge; semi-double petals; entire or serrated leaves.	AF WSK6	leaf	4 weeks	++
TC4	Aster purple (R.H.S. 87C, H.C.C. 38/2) with irregular dashes of white, single to semi-double petals; entire leaves.	ASP WSK3	leaf	6 weeks	++

R.H.S.: Royal Horticultural Society Colour Chart, London, 1966

H.C.C.: Horticultural Colour Chart, The Royal Horticultural Society, 1941

+: 1-5, shoots

++: 6-15, shoots

+++: 15 and above shoots.

Table 2
The survival rate of *Saintpaulia* in open frames compared with covered ones

Code of variety	Colour	Open frame		Enclosed frame	
		Number planted	Size of frame	Survival rate	Survival rate
B/P1	Gertian Blue	30	23 x 30 x 15 cm	10%	100%
B/P4	Violet-purple	40	46 x 31.5 x 52 cm	10%	100%
P1	Rhodamine Pink	30	23 x 30 x 15 cm	20%	100%

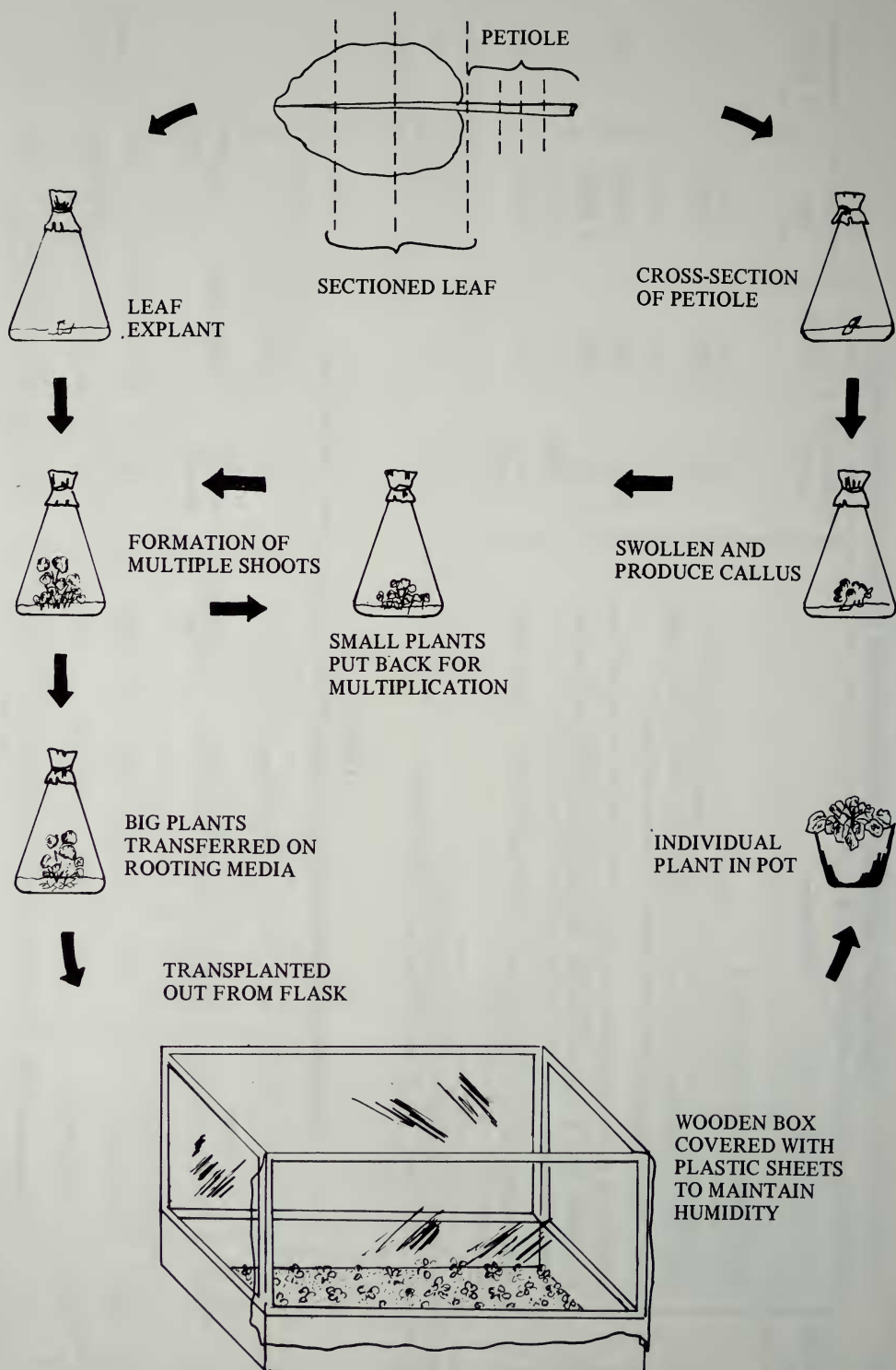


Fig. 1. Propagation Of Saintpaulia By Tissue-Culture Technique Pictorially Represented

Transplanting

Plantlets 2.5 cm in diameter with 6–8 leaves (Plate 2) were removed from the flask with long forceps. They were rinsed 2–3 times with distilled water to remove the attached agar and then soaked in a 0.5% Tersan 75 solution for about 5 minutes. The plantlets were transferred into sand-frames which were covered with a plastic sheet. They were incubated for 2 months and watered once a fortnight. So far all those transplanted into sand-frames which were covered have survived. In contrast, when open frames were used, at the earlier stages of our experiment, only 10–20% of transplanted plantlets have survived (Table 2). The size of the frames, however, did not appear to have a significant effect on the survival rate. After 2 months in the covered sand-frames (Plate 3) the plantlets were ready for transfer into individual pots.

Potting Stage

The mature plants from the sand-frames were potted individually in soil mixtures at the nursery. They grew well in a large variety of soil mixtures. A good mixture is obtained by mixing equal portions of topsoil, sand and peat. The plants were watered on alternate days and fertilised once a week with a concentration of NPK 75:60:60-p.p.m. Spraying with a mixture of 0.05% Rogor and of 0.15% of Captan 50 was carried out fortnightly.

Summary and Discussion

Our experiments showed that for *Saintpaulia*, leaf culture is preferable to petiole culture. The procedures are summarised in figure 1.

The cultures appeared to develop well on a variety of media. However, an overdose of growth regulators in the rooting medium tended to stimulate development of axillary shoots at the expense of root-development.

A crucial stage of propagation is the planting out of plantlets from flask. It appeared that most of them need to be incubated for about 1–2 months in an enclosed environment to enhance survival. The reason seems to be that the roots of the tissue-cultured plantlet cannot initially take on all their functions when planted in soil. Failure to replace water which was depleted rapidly through the leaves led to wilting and death. It is therefore necessary to keep the newly released tissue-cultured plantlets under heavy shade and in an enclosed environment until roots function fully. Fossard (1972) using scanning electron microscopy revealed that plants growing in culture have little or no crystalline wax on their leaves. This lack or low quality of crystalline wax on leaves increases transpiration and water-loss. The same may be the cause of rapid dessication of tissue-cultured plantlets when these are removed from their humid environment in the culture flask and placed in a drier condition.

Compared with the conventional leaf-cutting method, tissue-culture propagation has the following advantages:

- (1) very little space is required
- (2) growth rate is greater
- (3) new cultivars can be readily propagated



Plate 3. A sand-frame planted with *Saintpaulia*, P1, B/P2, B/P4 & B/P5, two months old. (Uncovered here to show details).



Plate 4. *Saintpaulia*, B/P4, from tissue-culture, grown in shade at the potting yard, with a compact rosette of leaves.



Plate 5. *Saintpaulia*, B/P4, propagated from leaf-cutting grown in shade at the potting yard with a loose rosette of leaves.

An interesting unexplained difference between plants derived from tissue culture and conventionally propagated ones has been observed. The tissue-cultured plants in general have a compact rosette of leaves with a short petiole (Plate 4) and the flower stalk is also short. Conventionally grown plants often have a loose rosette of leaves with a long petiole, and a long flower stalk (Plate 5). Tissue-cultured plants therefore are further enhanced for marketing purposes.

References

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